IMPAIRED RENAL POTASSIUM EXCRETION IN PATIENTS WITH SICKLE CELL DISEASE

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This thesis is dedicated to those individuals who generously sacrificed their time and energy to participate in this study: ED, RB, JG, SE, LC, and AE.

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INTRODUCTION

Sickle cell disease is one of the most frequently occurring hereditary conditions. Since 1949, when Pauling et al (1) described a different mobility of sickle hemoglobin on electrophoresis, much has been learned about the molecular structure of normal and abnormal hemoglobins. However, at the present time, relatively little is known about the pathophysiology of the clinical manifestations of the disease.

It is believed that the physical properties of the reduced Hb S molecule account for most of the clinical manifestations of sickle cell disease. Linear orientation of sickle hemoglobin molecules in the deoxygenated state is considered to be the cause of the sickle cell deformity. Factors which are known to cause linear polymer formation, and in turn, sickling of cells include low levels of oxygen tension (2), a drop in pH (3), and hypertonicity of the surrounding medium (4). The physiologic consequences of such changes are an increased blood viscosity secondary to clumping of sickled cells and resultant damage to the microvasculature. The actual clinical features of the disease may be related to these phenomena. Since the medullary area of the kidney is the site of generation of low pH and high osmolality, and possesses a low oxygen tension, it is not surprising that patients with sickle cell disease have significant, progressive impairment of renal function. The purpose of this study was to investigate renal tubular function in patients with sickle cell disease, with particular emphasis on their ability to excrete potassium.

LITERATURE REVIEW

Anatomical Changes

An understanding of the renal manifestations of sickle cell disease may be gained by reviewing the anatomical changes observed in the kidneys of such patients.

The most consistent finding on microscopic examination of the glomeruli of the kidneys of sickle cell patients is overall enlargement, with dilatation of glomerular capillaries (5). Bernstein and Whitten (1960) examined fifteen autopsy cases of children (ages 1-15 years) wich sickle cell disease. In those children under the age of two the glomeruli appeared normal except for mild vascular congestion and dilatation of the vascular loops. In the older children the glomeruli were more congested with striking glomerular enlargement, particularly of the juxtamedullary glomeruli. This enlargement was due primarily to an increase in glomerular vascularity. Focal areas of ischemia and fibrosis were common. Pitcock et al (6) reported on renal biopsies performed on patients 17 to 27 years of age who had Hb SS but no evidence of clinical renal disease except for a urinary concentrating defect. They noted stainable iron in the glomerular epithelial cells of both parietal and visceral type, usually in association with an increase in mesangial matrix, and with electron dense material in mesangial cells. In some cases there was focal fusion of foot processes and occasional small granular deposits in glomerular basement membrane. The significance of these findings are questionable, however, since most evidence suggests that patients with sickle cell disease rarely develop proteinuria,

the nephrotic syndrome, or progressive glomerulonephritis (7).

There are occasional reports of the presence of the nephrotic syndrome in patients with sickle cell disease. However, it has not always been possible to exclude other etiologic factors in these cases. McCoy (8) studied three patients with nephrotic syndrome who were found to have electron-dense deposits in the mesangium and glomerular basement membrane. He found that these deposits stained for iron, and he postulated that they represented protein-iron complexes originating in the circulation that had been phagocytized by glomerular mesangial cells. He further postulated that these complexes were responsible for the development of the nephrotic syndrome. Walker (9) studied renal biopsy specimens of SS patients. Four had normal renal function studies, 3 had proteinuria, 2 had uremia. Some of the specimens he reviewed had lesions indistinguishable from those of a proliferative glomerulonephritis, however, the patient with the lowest creatinine clearance in his group had normal glomeruli suggesting that in these patients renal function tests did not reflect the degree of pathological glomerular disease.

More recently, Pardo et al (10) reported an immune complex glomerulonephritis in seven patients with sickle cell anemia and proteinuria. One or more findings of the nephrotic syndrome, hypertension, hematuria, and renal insufficiency were found in more than half of the patients. All patients had membranoproliferative glomerulonephritis of varying degree. Immunoflourescent studies revealed IgG and C3 in a granular pattern along the basement membrane and the mesangium of all patients studied. Renal tubular antigen was localized in the same pattern as immunoglobulins and C3 in two of

the subjects studied. On the basis of these findings, these authors suggested that the membranoproliferative glomerulonephritis associated with sickle cell anemia is a result of an autologous immune complex disorder. The suggestion is made that tubular defects could lead to release and subsequent sensitization to the tubular antigen, and that these renal tubular antigen-antibody complexes are deposited in the glomeruli and cause the observed lesions. Antibody to renal tubular antigen was detected in the serum of two subjects in this study. It should be emphasized that the significance of these findings is questionable because of the rarity of reported cases of glomerulonephritis in patients with sickle cell disease.

The most striking changes in renal morphology in patients with sickle cell disease are found in the medullary area. There is considerable dilatation of the vasa recta, with extravasation of blood into the surrounding tissue (7). Microangiographic studies have demonstrated amputation of the vasa recta secondary to sickling of red blood cells in the hypertonic medullar interstitium (11). Even those vessels which are present are often abnormal, being dilated, forming spirals, or ending bluntly. These vascular changes are associated with interstitial infarcts, scarring, tubular atrophy of varying degrees, and papillary necrosis. Similar changes have been reported by Mostofi, who noted foci of tubular degeneration, edema of the papillary stroma, and patchy interstitial fibrosis in kidneys removed from sickle cell patients with hematuria (12). The papillary necrosis seen in patients with sickle cell disease is referred to as the "medullary form" (13,14) and is a more limited necrosis than traditional papillary necrosis in which the entire papilla is often

sloughed. This lesion is thought to be secondary to the ischemic vascular changes.

Functional Changes

Renal Plasma Flow and Glomerular Filtration Rate: Numerous studies performed in children with sickle cell anemia have documented an increase in glomerular filtration rate and total renal blood flow, associated with a fall in filtration fraction when compared to age-matched controls (15,16). By young adulthood (20-30 years) renal blood flow and GFR have returned to normal or are absolutely reduced, and in older patients these parameters are more often subnormal (16). Thus, the published data suggest that the elevated GFR in children with sickle cell disease begins to decline by the second and third decade of life. However, the precise time course of the decline and its relationship to clinical activity are uncertain. In all age groups it has been shown that filtration fraction is decreased, i.e. there is a disproportionate increase in effective renal plasma flow in proportion to glomerular filtration rate (16).

The causes of the increased RPF and GFR in young patients with sickle cell anemia are unknown. The fact that correction of the anemia by prolonged transfusion of normal cells does not correct these abnormalities has been interpreted to mean that the anemia per se is not the underlying cause (17). Moreover, there is no apparent relationship between hematocrit and the GFR in the sickle cell patient (18). Although the suggestion has been made that a primary increase in renal blood flow secondarily causes the increased GFR, this has not been proven. With time progressive glomerular ischemia, fibrosis and eventual glomerular obliteration results in the observed decline

in renal blood flow and GFR seen in later life.

Urinary Concentration: Inability to maximally concentrate the urine is the most consistently observed and best studied renal functional abnormality in patients with sickle cell disease. The defect is present in homozygotes, patients with Hb SC, and Hb S-thal, and heterozygotes and appears to be related directly to the presence of sickle hemoglobin (4). The concentrating defect can be detected as early as the first year of life, and becomes progressively more severe. In young patients concentrating ability can be restored to normal after multiple blood transfusions. However, in older patients (greater than 15 years) blood transfusions are ineffective in reversing the concentrating defect (19), implying structured damage to the medulla.

Patients with sickle cell disease are able to dilute their urine normally but are usually unable to concentrate above 700 mosm/kg. Maximally concentrated urine is produced by extraction of water from tubular fluid passing through hypertonic medullary interstitial tissue. Medullary hypertonicity results from reabsorption of sodium chloride and urea into the renal medulla by a mechanism which depends on countercurrent flow of tubular fluid and blood through the loops of Henle and vasa recta respectively (20). Furthermore, the differential permeabilities of the loop of Henle and collecting duct are necessary for these processes to occur.

It is believed that sodium chloride accumulation in the medullary interstitium is largely a result of active transport of chloride from the thick ascending loop of Henle. Hypotonic tubular fluid is then presented to the distal tubule and collecting duct where water is reabsorbed. As fluid passes more distally the tubule becomes

permeable to urea, and this substance is reabsorbed from the collecting duct into the surrounding medullary tissue (20). Recent evidence suggests that reabsorption of urea into the medullary interstitium enables NaCl to be passively reabsorbed by the thin ascending limb of the loop of Henle, and that this process is necessary for concentrating the urine above 600 mosm/kg (21).

Several authors have postulated that the inability of patients with sickle cell disease to maximally concentrate their urine is a result of defective NaCl reabsorption in the loop of Henle (4). However, under conditions of solute loading with mannitol or hypertonic saline, it has been shown that these patients are able to increase negative free water clearance (T^cH_2O) appropriately with increasing solute loads (22). Normal T^cH_2O depends on NaCl reabsorption from the loop with resultant water reabsorption from the collecting duct, and does not depend on medullary accumulation of urea (23). This implies that sicklers have a normal ability to reabsorb increasing amounts of NaCl, and that a defect in this process is not responsible for their inability to attain a normal, maximally concentrated urine.

The unique situation in sickle cell patients, i.e. inability to maximally concentrate their urine associated with normal T^cH_2O and normal urinary dilution is most likely a consequence of the decreased blood flow in the vasa recta (7). As previously mentioned, Perillie and Epstein have shown that red blood cells containing Hb S become sickled when immersed in hypertonic solutions (4). A similar phenomen <u>in vivo</u> has been postulated as sickle cells enter the hypertonic medulla. The resulting sickling leads to increased blood viscosity and decreased medullary blood flow, which has been

by microangiographic studies (11). The result then, is a "functional papillectomy" (7). Papillary ischemia results in decreased function or obliteration of the juxtamedullary nephrons with long thin loops of Henle, since it is these nephrons that populate the areas of abnormal blood flow (7). It appears that the juxtamedullary nephrons are not necessary for elaboration of a maximally dilute urine. However, since the formation of maximally concentrated urine depends on urea reabsorption from the collecting duct and subsequent passive (as well as active) NaCl transport out of the thin ascending limbs of these juxtamedullary nephrons, their destruction results in an inability to generate maximum Uosm greater than 600 mosm/Kg (21). Studies in rats support this concept. Lief et al have shown that $T^{c}H_{2}O$ is normal during solute loading in papillectomized rats in spite of a severe defect in Uosm Max (24).

The hypothesis put forth then, is that decreased perfusion of the renal papilla results in a functional papillectomy which is reversible by transfusion in the early stages. The loss of the papilla does not interfere with the bulk reabsorption of solute free water, since this depends on intact functioning of the thick ascending loops of Henle and cortical collecting ducts in the outer medulla and cortex. With time, chronic ischemia of the renal papilla leads to irreversible damage so that transfusions will not correct the defect.

Urinary Acidification: In recent years it has been found that patients with sickle cell disease have an incomplete distal renal tubular acidosis (RTA) (25,26,27). The finding of defective urinary acidification is not surprising since final urine acidification takes place in the collecting duct, an area which is often damaged in the

sickle cell kidney.

Acidification has been studied in West Indian patients by Goossens et al (25), and Ho ping Kong and Alleyne (26), and in American Blacks by Oster et al (27) using the short ammonium chloride acidification test (28). In the West Indian studies minimum urinary pH after acid loading was significantly higher and titratable acidity (TA) was significantly lower in patients with Hbs SS and SC, compared to subjects with Hbs SA and AA. NH_4^+ excretion did not differ among any of the subjects studied. A correlation between severity of the concentration defect and severity of the acidification defect was also noted. These studies demonstrate that patients with sickle cell disease have an "incomplete distal RTA", that is, there is an inability to appropriately lower urine pH when stressed, but in the basal state systemic acidosis is not present.

Oster et al (27) studied 20 Black Americans with sickle cell disease, and compared them to patients with Hb AS and Hb AA. They also evaluated maximum urine to blood pCO_2 gradients during $NaHCO_3$ loading. They found that 6 of 20 patients with sickle cell disease were unable to lower their urine pH to 5.3 or less after NH_4Cl administration, and that the mean urine pH was higher than controls or Hb AS subjects. Hb SS subjects manifested lower excretion of TA and net acid in comparison to controls. There were no clinical, hematological, renal or acid-base differences between the Hb SS subjects with and without a normal response to acid loading. Their study also demonstrated that the bicarbonate loading test may be more sensitive in uncovering acidification abnormalities, since 5 of 7 patients with sickle cell disease with normal responses to ammonium chloride had abnormal urine-blood pCO_2. The results of this

study differ somewhat from the West Indian investigations which demonstrated that almost every patient studied was incapable of a normal response to acid loading.

It is clear that a mild form of incomplete distal RTA is present in many patients with sickle cell disease. Although the mechanism for this abnormality remains unknown, it is conceivable that it could result from collecting duct dysfunction secondary to the disturbances in papillary blood flow found in these patients. The clinical significance of this problem is not clear. Studies have shown that in vitro acidosis enhances the sickling of erythrocytes from patients with sickle cell disease (2) and several authors have reported precipitation of painful crises by NH_4Cl or acetazolamide induced acidosis (29,30). Others have also reported that metabolic acidosis is a major precipitating factor of painful crises (31). However, most of this data stems from reports of isolated cases and further studies have not shown a consistent relationship between systemic acidosis and painful crisis. Furthermore, the observation that these patients are not acidemic in the basal state as a result of this defect in acid excretion makes it unlikely that an incomplete RTA is clinically significant in these patients.

Hematuria: One of the more dramatic manifestations of sickle cell disease is hematuria. It is found in all persons who possess the gene for sickle hemoglobin, either in the homozygous or heterozygous state and painless, gross hematuria is a common finding (12,32). As discussed above, patients with sickle cell disease have reduced perfusion of the renal medulla, probably secondary to increased sickling of cells in this area and subsequent increased blood viscosity. Furthermore, these patients have an overall increased renal blood

flow. This combination of events -- increased renal blood flow with reduced medullary blood flow has been offered as an explanation of the frequent occurrence of hematuria in these patients (7,33). It has been found in the rat and the dog, that efferent vessels from juxtamedullary glomeruli divide to form the vasa recta and peritubular capillaries of juxtamedullary nephrons; furthermore they also form the nutrient vessels for the renal pelvis. These latter vessels have been found to be engorged and dilated in patients with sickle cell disease (11). It is suggested that engorgement of pelvic vessels could result from shunting of efferent arteriolar blood flow into pelvic mucosal capillaries thereby causing rupture of these vessels with subsequent hematuria (7,33).

BACKGROUND AND PURPOSE

Several conclusions can be made after reviewing the literature on the renal complications of sickle cell disease. Many of the anatomical lesions in the kidneys of these patients appear to be the result of vascular occlusive changes, which are secondary to sickling of red blood cells. The most striking changes occur in the medullary region and correlate with the abnormalities of the vasa recta (11). Characteristically, the pathologic changes consist of patchy interstitial fibrosis, interstitial edema, tubular atrophy, and papillary necrosis (12). The renal functional changes can best be understood when viewed in the context of these anatomical changes. Thus, we have seen that the sickle cell patient behaves as though he were "functionally papillectomized'. (7)

Finkelstein and Hayslett (34) have previously examined the contribution of medullary structures to urinary concentration and potassium and hydrogen ion homeostasis in rats who had undergone a surgical papillectomy which resulted in the loss of the terminal portion of the collecting ducts and interruption of loops of Henle of juxtamedullary nephrons. They found that papillectomized rats exhibited a severe urinary concentrating defect and a reduction in total acid excretion following ammonium chloride loading that was primarily due to decreased ammonium excretion. The ability to acidify the urine was not impaired by papillectomy. The papillectomized animals also demonstrated a markedly impaired capacity for potassium (K) excretion following potassium loading. These observations in the papillectomized rat (impaired concentrating and acidifying ability and impaired potassium excretion) are in agreement with our

current concepts of the function of the distal nephron.

The surgically papillectomized rat kidney is not unlike the kidneys of patients with sickle cell disease in whom occlusion of the vasa recta from the sickling process often results in the ablation of the papilla. Thus, it is not surprising that patients with sickle cell disease exhibit defects in urinary concentration and acidification. However, no data have been reported concerning the renal handling of potassium or the integrity of the renin-aldosterone axis in these patients. Although abnormal potassium excretion has not been reported previously as a complication of sickle cell disease, such a defect has not been specifically looked for. On the basis of the renal histopathologic changes affecting the medullary interstitium such a defect in potassium excretion might be expected. Recent studies summarized by Giebisch (35) have shown that greater than 90-95% of the filtered potassium load is reabsorbed by the early distal tubule and most of the potassium appearing in the final urine is the result of secretion by the distal and collecting tubular cells. Thus, in sickle cell disease where the collecting tubules are selectively injured by ischemic damage to the medullary and papillary interstitium, one might expect an impaired ability to secrete potassium as has been described by Finkelstein and Hayslett in the papillectomized rat. Such a defect may assume clinical significance in the sickle cell patient during crisis where intravascular hemolysis can acutely present an increased load of potassium to the distal nephron for secretion.

Hyperkalemia and impaired renal potassium excretion has also been described in association with the syndrome of hyporeninism-hyperaldosteronism (36). This syndrome has been observed in two

clinical settings: patients with diabetes mellitus and patients with interstitial nephritis of diverse etiology (6). In the latter group it has been postulated that there is selective damage to the juxtaglomerular apparatus with resultant hyporeninism. Thus, in patients with sickle cell disease one might anticipate a defect in potassium excretion which could be secondary to either a defect in the renin-aldosterone axis or to primary tubular damage.

It was the purpose of the present study to examine tubular function in subjects with sickle disease with special emphasis on the renal tubular handling of potassium and the integrity of the renin-aldosterone axis.

Methods

Six patients with sickle cell disease (4 Hb SS, 1 Hb SC, 1 Hb S-thal) were admitted to the Clinical Research Center at Yale-New Haven Hospital for a total of seven days per patient. Brief clinical histories and other pertinent laboratory data are presented in the appendix. Clinical and hematological data are presented in Table 1. Patients ranged in age from 19 to 37 years. All were anemic and possessed at least 50% Hb S at the time of the study. Baseline renal function studies are shown in Table 2. Serum electrolytes, BUN and creatinine were normal in all subjects. Prior to study all patients were placed on a three gram sodium, 2 gram potassium diet which was continued throughout the study period except as specified below.

Day 1: Administration of ACTH. Patients were placed at bed rest for one hour after which baseline bloods for plasma cortisol and plasma aldosterone concentrations and plasma renin activity were

drawn. ACTH (Cortrosyn) 0.25 mg was then given intramuscularly and repeat bloods were drawn one hour after administration.

Day 2: Sodium Sulfate Infusion and Determination of Inulin and PAH Clearance. Patients remained fasted after midnight of Day 1 until after the $NaSO_4$ study was completed. At 7 a.m. a small polyethylene catheter was inserted into one antecubital vein for collection of blood samples. Priming doses of inulin (40 mg/kg body weight) and para-aminohippurate (PAH) (12 mg/kg body weight) were administered intravenously, and these were followed by sustaining infusions of inulin and PAH in 0.9% sodium chloride at a rate of 0.5 ml/min. Subjects remained supine except to void. A water diuresis was initiated with an oral water load (15 ml/kg) and the volume of each voiding was replaced by deionized water orally to maintain a high rate of urine flow. After a 45 minute equilibration period, two control urine volumes were collected. A 0.12 M solution of sodium sulfate (dose = 1.8 mMoles/kg body weight) was then infused over two hours. Urines were collected every thirty to forty minutes for a total of six hours commencing with the initiation of the sodium sulfate infusion. Three bloods were drawn during the control period and then at one hour intervals thereafter until the end of the study. All bloods were analyzed for sodium, potassium, inulin, and PAH.

Day 3: Water Deprivation Study. Patients were fasted after 8 p.m. of the previous night. Upon arising at 7 a.m. and again at 10 a.m. urine samples were obtained for determination of urine osmolality (Uosm). 500 mU of aqueous vasopressin was infused intravenously from 10-11 a.m. and the next spontaneously voided urine (voided within 2 hours) was collected and Uosm determined.

Day 4: Potassium Chloride Infusion. All patients remained fasted after 12 midnight of the preceding night. At 7 a.m. catheters were inserted into each antecubital vein and a water diuresis was initiated and maintained as described in the section on sodium sulfate. Two control urines were collected by spontaneous voiding. 0.75 mEq of potassium chloride per kilogram dissolved in 400 ml of 1/4 normal saline was then infused at a constant rate over 120 minutes. Most patients experienced transient discomfort at the site of the infusion which passed within 30 minutes. Continuous electrocardiographic monitoring revealed no changes of hyperkalemia. Two control bloods were obtained after 90 minutes of supine posture for determination of plasma aldosterone concentration and plasma renin activity before the KCl infusion. Bloods for aldosterone and renin were subsequently obtained at 60, 120, 240, and 360 minutes after beginning the KCl infusion. Plasma potassium, sodium and creatinine concentrations were determined on at least three occasions during the control period and every 30-60 minutes thereafter. All blood removed was replaced quantitatively by an equal amount of normal saline intravenously. Urines were collected every 30-45 minutes for a total of six hours starting with the potassium chloride infusion and analyzed for sodium, potassium, and creatinine. Patients remained supine except to void.

Day 5: Ammonium Chloride Loading Study. Urinary acidification was studied using the short ammonium chloride test of Wrong and Davies (28). Patients were fasted after 12 midnight of the preceding night. At 7 a.m. a polyethylene catheter was inserted into an antecubital vein for blood sampling. Enough deionized water was administered orally to ensure voiding every 30-45 minutes. Following

the collection of two control urines, ammonium chloride at a dose of 0.1 grams/kg in gelatin capsules was given orally over 90 minutes. All urines were then collected under mineral oil for eight hours and analyzed for ammonium, titratable acid, sodium, potassium and creatinine. Urinary pH was determined within 5 minutes of obtaining the specimens. Bloods for sodium, potassium, and creatinine were obtained during each control period and then hourly for remainder of the study. Venous blood was obtained during each control period and then at 2, 4, 6, and 8 hours after ammonium chloride for determination of pH, pCO_2 and bicarbonate.

Day 6: Furosemide and Volume Depletion. To determine the renal response to furosemide and the integrity of the renin-aldosterone axis, patients were first placed on a 500 mg sodium, 2 gram potassium diet. After obtaining two baseline urine specimens, each subject was given 40 mg of furosemide orally at 8 a.m. and urine specimens were collected individually until 3 p.m. Urine voided from 3 p.m. to 8 a.m. on day 7 was pooled to determine net sodium balance. Each sample was analyzed for sodium, potassium and creatinine. Bloods were obtained at 8 a.m., 11 a.m. and 1 p.m. and similarly analyzed. At 6 p.m. subjects were given a repeat dose of 40 mg of furosemide orally. Weights were obtained at 8 a.m., 6 p.m. and 8 a.m. of day 7. At 8 a.m. on day 7, after the patients had been supine overnight, blood was drawn for aldosterone and renin determination. The patients then ambulated for 2 hours and repeat bloods for aldosterone and renin were drawn. At the completion of the study the subjects received appropriate oral salt replacement.

Controls

Five healthy, age-matched volunteers studied in the same fashion as earlier described served as controls for the sodium sulfate and potassium chloride studies. Six subjects served as controls for the ammonium chloride study, and 9 for the furosemide study. Control data for urinary concentrating ability and cortrosyn study have been previously published. (45,46).

Signed informed consent was obtained after care had been taken to explain fully to the volunteers the details of the procedures and any potential risks involved. No adverse effects occurred.

ANALYTICAL METHODS

Urinary osmolality was determined on Advanced Osmometer Model 3W (Advanced Instruments, Inc.) by the method of freezing point depression. Urinary pH was determined on an Acid-Base Analyzer, type PH M 71B (Radiometer Electronic Measuring Instruments). Sodium and potassium were measured by flame photometry on a model IL 143 Digital Flame Photometer (Instrument Laboratory, Inc., Lexington, Mass.) with an internal lithium standard. Creatinine was determined by a continuous flow modification of the Jaffe technique (37). Titratable acidity was measured by titrating urine specimens to the pH of a reference buffer solution with 0.1 N NaOH. Ammonia was determined by the method of Conway (38). Inulin was determined by the anthrone method adopted for automated analysis (39) and PAH by the method of Goldring and Chasis (40).

Blood for aldosterone and plasma renin activity was collected in chilled syringes containing EDTA and promptly centrifuged at 4 degrees centigrade. Plasma renin activity in blood samples were determined by a modification of the radioimmunoassay technique described by Haber et al (41). Plasma aldosterone concentration was determined by a modification of the radioimmunoassay technique described by Bayard, et al (42), utilizing ammonium sulfate precipitation of bound antigen in the assay as described by Mayes, et al (43).

CALCULATIONS

In the statistical analysis of the data observations during control periods were averaged and compared with observations after drug administration (paired t-test). Changes in sickle cell subjects were compared with changes in control subjects using the Students t-test.

RESULTS

Glomerular Function. There was no evidence of significant glomerular dysfunction in any of the sickle cell patients. None had proteinuria, hypertension or hematuria. The inulin clearance (C_{IN}) was normal or elevated in 5 out of 6 patients and mildly reduced in one (Table III). Normal values for C_{IN} and C_{PAH} in age matched controls are 127 and 655 respectively (16). The mean C_{IN} for the patients with sickle cell disease was 122±13 ml/min. Renal plasma flow (C_{PAH}) was normal or elevated in all 6 patients with sickle cell disease with a mean value of 792±77 ml/min. Filtration fraction ranged from 0.11 to 0.20 with a mean value of 0.16±0.01. The normal value for filtration fraction in a group of age matched normal controls was 0.19 (16).

Tubular Function. Urinary Concentrating Ability: All six sickle cell patients had a severe urinary concentrating defect (Table IV). The mean Uosm after 12 hours of water deprivation was 389±13 mosm/kg, and increased to only 422±7 mosm/kg following vasopressin infusion. Maximum urine osmolarity in 31 age matched controls was 1,109±22 mosm/kg ($p<0.001$) after a similar period of water deprivation (44).

Ammonium chloride loading. Baseline serum bicarbonate and chloride concentrations and venous pH were normal in all sickle cell patients. Following ammonium chloride loading venous bicarbonate fell from 24±.47 mEq/L to 19±0.7 mEq/L in the sickle cell patients and from 26±0.5 mEq/L to 20±0.5 mEq/L in the controls. Venous pH fell from 7.39±0.01 to 7.34±0.01 in the patients with sickle cell disease and from 7.35±0.01 to 7.27±.01 in the controls (Table V). Urine pH fell to less than 5.3 in only one of six patients with sickle cell disease, with a mean decrease of the group to 5.52±.10.

This was significantly higher than minimum urinary pH in 6 controls in whom urinary pH after acid loading was 5.0±.10 ($p<0.01$). In all of the controls urine pH fell to 5.3 or less (Table VI).

Maximum excretion of titratable acid in sickle cell patients (42± 4 μeq/min) was significantly lower than in controls (96±11 μeq/min) ($p<0.001$). Maximum ammonia excretion in sickle cell patients (50±8 μeq/min) was also significantly less than controls (72±8 μeq/min) ($p<0.005$). Net acid production was lower in the sickle cell patients ($p<0.005$). Ammonia accounted for a higher percentage of net acid production in the sickle cell patients compared to the controls (54±3% compared to 42±2%, $p<0.02$) (Table VI). When ammonia excretion was plotted against urine pH, the individual values for the sickle cell patients were lower than those of controls, indicating that the lower values for ammonia excretion in sickle cell patients could not be attributed to higher urinary pH.

Figures 1-3 illustrate pH, titratable acid and ammonia excretion during and after acid loading. The values for each of the sickle cell patients are plotted individually, and the range of the values for the controls is shown by the shaded area. There was no overlap in titratable acid excretion between the two groups during the entire study. Urine pH was significantly different between the two groups from hours two through six of the study ($p<.01$). The differences in ammonia excretion, while not as marked as with titratable acid, persisted throughout the study.

Sodium Sulfate Infusion. The response to sodium sulfate infusion was markedly impaired in all sickle cell patients (Figure 4, Table VII). Baseline urinary potassium excretion in the sickle cell group

(37±5 μeq/min) was significantly lower ($p<0.01$) than in controls (91±4 μeq/min). The maximum increment in potassium excretion above baseline in sickle cell patients (20±4 μeq/min) was also significantly less than in controls (107±20 μeq/min) ($p<0.001$).

In figure 4 the individual values for potassium excretion (U_KV) are plotted for the six hour time period beginning with the start of the sodium sulfate infusion. The sickle cell patients demonstrated only a very slight increase in U_KV in response to sodium sulfate in contrast to the controls, in whom potassium excretion reached a maximum at $1\frac{1}{2}$ hours after the infusion was begun. The differences in potassium excretion between the two groups are highly significant at each individual time point ($p<0.01$).

The increase in sodium excretion following sodium sulfate was similar in controls and sickle cell patients. Both groups reached a peak $U_{Na}V$ of approximately 800 μeq/min two hours after the infusion was begun and in both sodium excretion returned to baseline by the end of six hours.

There was no difference in plasma potassium or sodium concentrations between the two groups at any time during the study.

Potassium Chloride Infusion. Baseline potassium excretion was lower in the sickle cell patients (33±4 μeq/min) compared to controls (77±15 μeq/min) ($p<0.01$). Baseline plasma potassium concentrations were similar in the two groups. The maximum increase in potassium excretion following potassium chloride loading was significantly lower in the sickle cell group than in the controls (114±14 μeq/min compared to 353±41 μeq/min; $p<0.001$). The percent of the potassium load excreted over the five hour experimental period was 28±5% in

the sickle cell patients compared to 61±7% in the controls ($p<0.01$). Table VI, Figure 5 is a plot of the urinary potassium excretion during and after the potassium chloride infusion. Although the sickle cell patients demonstrated some increase in U_KV above baseline, compared to the controls the response is markedly impaired.

Despite the marked impairment in potassium excretion observed in the sickle cell patients, the increase in plasma potassium concentration was similar in both groups. Figure 6 shows there was no difference in the plasma potassium concentrations between sickle cell patients and controls at anytime during the potassium chloride study.

Furosemide and Volume Depletion. Baseline potassium and sodium excretion were not significantly different between the sickle cell patients and the controls. Maximum potassium excretion after furosemide was 99±13 μeq/min in the sickle cell patients and 203±20 eq/min in the controls ($p<0.001$) (Table IX). In figure 7 the values of U_KV for each sickle cell patient are displayed. The normal control range is shown by the shaded area. Potassium excretion increased dramatically in the controls with peak excretion occurring by the end of the third hour. Only two sickle cell patients (RB and LC) increased U_KV to the same range as the controls.

Sodium excretion followed a similar pattern to that observed with potassium excretion (Table IX, Figure 8). Five out of six sickle cell patients demonstrated a blunted natriuretic response to furosemide but a statistically significant difference was not achieved because of the exaggerated natriuretic response of patient RB. When this patient is omitted the peak $U_{Na}V$ was significantly

less than controls ($p < 0.001$).

Plasma aldosterone and plasma renin activity were determined at the end of this part of the study to evaluate their response to volume depletion. Table X compares baseline plasma renin activity and plasma aldosterone concentration before and after volume depletion in the sickle cell patients. There was a significant increase in plasma aldosterone from 9.1±84 ng/100 ml to 24.3±5 ng/100 ml with volume depletion and a further increase to 78±30 ng/100 ml was achieved after two hours of ambulation. Plasma renin activity increased similarly from .74 ng/ml of angiotension II generated per hour to 6.75±1.9 ng/ml/hr and then to 14.08±4.3 ng/ml/hr. These results are similar to values previously reported by us in healthy controls (62).

Administration of ACTH (Cortrosyn). The results of administration of synthetic ACTH to the sickle cell patients are shown in Table XI. All patients demonstrated an increase in plasma cortisol and plasma aldosterone in response to cortrosyn. Plasma aldosterone rose from a mean value of 10.7±1.5 ng/100 ml to 36.5±18 ng/100 ml. Plasma cortisol rose from a mean value of 11.5±1.4 µg/100 ml to 25±7.7 µg/100 ml. The mean increment in plasma aldosterone was 3.2 fold, while that for cortisol was 2.2 fold. These results are in agreement with reports in the literature of normal subjects in whom plasma aldosterone rose 3.4 fold and plasma cortisol rose 2.8 fold in response to similar doses of synthetic ACTH (45,46).

DISCUSSION

This study documents the presence of severe tubular dysfunction in patients with sickle cell disease despite normal or elevated glomerular filtration rate and renal plasma flow. All six patients studied manifested a severe defect in urinary concentrating ability that was resistent to administration of exogenous vasopressin. Abnormal urinary acidification was also observed in all six sickle cell patients following ammonium chloride administration. Five out of six patients were unable to lower their urine pH to less than 5.3 and all six patients demonstrated a blunted maximum increase in urinary excretion of titratable acid and ammonia. Since serum chloride and bicarbonate concentrations and venous pH were normal in all, these patients can be assumed to have an incomplete distal renal tubular acidosis. Other authors who have previously examined urinary acidification in patients with sickle cell disease (25,26,27) have also reported higher urinary pH and lower titratable acid excretion in sickle cell patients after ammonium chloride. However, none of these previous studies have reported significantly lower ammonia excretion in sickle cell patients when corrected for urinary pH. In patients with incomplete renal tubular acidosis (RTA) ammonium characteristically constitutes a greater than normal fraction of urinary acid both before and during induced acidosis (47). In our patients ammonia also represented a greater fraction of total acid produced but absolute excretion was lower than that observed in controls. The finding of diminished ammonia excretion, even after correction for urine pH, is in contrast to other reported cases of incomplete RTA, where ammonia excretion is normal or in-

creased (28,47). The decrease in NH_4^+ excretion is, however, consistent with previously described histopathologic changes in sickle cell patients where renal medullary and papillary ischemia results in interstitial scarring and tubular atrophy with varying degrees of distal and collecting tubular dysfunction. Since ammonia excretion and final acidification occur primarily in the collecting tubules (20), the incomplete RTA seen in these patients is not unexpected. Since maximum urinary concentration is also dependent upon normal collecting duct and loop of Henle function, and since these structures are selectively injured in the sickle cell kidney, it is not surprising that these patients also demonstrate a severe vasopressin-resistant defect in concentrating ability.

All of our patients with sickle cell disease demonstrated abnormal urinary potassium excretion in response to intravenous sodium sulfate and potassium chloride, as well as to oral furosemide. Renal potassium handling has not previously been examined in patients with sickle cell disease. However, since potassium excretion primarily reflects secretion in the distal nephron (distal tubule and collecting duct) (35), impairment of this aspect of tubular function might be expected in sickle cell patients.

Potassium secretion by the distal and collecting tubules is thought to be a passive process, with potassium diffusing from a region of high concentration (intracellular potassium is maintained at a high level by an active pump located on the pericapillary side of the distal and collecting tubular cells (35) (Figure 9, step 1). The passive diffusion of potassium from cell to lumen is also favored by the electrical gradient, lumen 50 millivolts negative with respect to cell (49) (step 2). The presence of adequate amounts of sodium in the distal and collecting tubular fluid is responsible for the

generation of the electromotive force that drives potassium from tubular cell to lumen (50). Since sodium permeability is greater than that of chloride, reabsorption of sodium causes the lumen to become slightly electronegative, and this in turn serves as a driving force for potassium secretion (step 3). The transcellular movement of potassium from pericapillary to lumenal side in exchange for sodium is also strongly modulated by aldosterone (51).

Defective potassium excretion could, therefore, be the result of decreased delivery of sodium to the distal nephron, a defect in aldosterone secretion, or a primary defect in tubular secretion of potassium. Differences in sodium delivery to distal sodium-potassium exchange sites cannot account for the impairment in urinary potassium excretion observed in our sickle cell patients. During the seven day balance period when dietary intake of sodium and potassium remained constant, daily urine sodium excretion remained in the normal range. Following both sodium sulfate and potassium chloride infusions, sodium excretion rose similarly in both control and sickle cell groups, yet urinary potassium was markedly decreased in the latter.

It is well known that aldosterone modulates potassium excretion by the kidney (51,52). Impaired potassium secretion with resulting hyperkalemia has frequently been recognized as secondary to hypoaldosteronism, and hyperaldosteronism is associated with excessive urinary losses of potassium (53). The effect of aldosterone on potassium transport is probably independent of its effect on sodium excretion - Williamson, et al have shown that if adrenalectomized rats are treated with Dactinomycin (to prevent RNA synthesis),

the sodium reabsorption response of aldosterone is blocked, but the kaliuretic effect is intact (54,55). Several reports have appeared in the literature attributing the development of hyperkalemia and decreased potassium excretion in patients with renal disease to the syndrome of hyporeninemic-hypoaldosteronism (56,57). The mechanism for this specific defect is not known, however, it has been suggested that an abnormality of renin production may be the result of damage to the juxta-glomerular apparatus acquired during the course of the renal disease (56).

Because of the important role of aldosterone in renal potassium handling we attempted to determine whether the impairment in potassium excretion observed in the sickle cell group could be attributed to a defect in the renin-aldosterone axis. Such a defect might be expected because of the known association of hypoaldosteronism and renal diseases resulting from interstitial damage.

Previous studies have shown that acute administration of ACTH is a potent stimulus to both cortisol and aldosterone secretion (45). The plasma cortisol and aldosterone responses to cortrosyn in all six sickle cell patients were similar to values previously reported in the literature for control subjects (45,46). The integrity of the renin-aldosterone axis was also examined following volume depletion, a potent stimulus to aldosterone secretion. A normal increase in both plasma renin activity and plasma aldosterone concentration was observed. These results demonstrate a normally functioning renin-aldosterone axis in sickle cell patients and exclude hypoaldosteronism as the cause of the impaired potassium excretion.

Defective tubular secretion of potassium in the absence of significant renal impairment or hypoaldosteronism has only rarely been reported (57,58,59,60,62). Healy et al (61) described the first such case of unexplained hyperkalemia in a young boy with normal adrenal and renal function but with an inability to augment renal potassium excretion in response to flourohydrocortisone, sodium sulfate, or acetazolamide, all of which are known to markedly enhance renal tubular potassium secretion. Subsequently, three more case reports have appeared describing a similar tubular defect in potassium excretion (57,58,60). To examine this possibility in sickle cell patients all subjects received a standard sodium sulfate infusion. This is known to directly enhance tubular secretion of potassium by enhancing delivery of sodium to the distal sodium-potassium exchange sites, while at the same time increasing the lumenal negativity because of the impermeant sulfate anion. Despite a similar increase in sodium excretion the increase in potassium excretion in sickle cell patients was markedly blunted, suggesting a primary defect in tubular secretion of potassium. Most recently DeFronzo et al have documented a similar defect in patients with systemic lupus erythematosis (SLE) (62). Since other investigators have shown that many patients with SLE have a prominent interstitial nephritis that is associated with the deposition of antibodies in the tubular basement membranes and the surrounding interstitium (63,64), it is interesting to speculate that the hyperkalemia is the consequence of tubular dysfunction secondary to these immune globulin deposits. Since sickle cell patients have also been shown to have a prominent interstitial nephritis, it is possible that a similar immunologic process is present.

Our patients also demonstrated marked impairment in renal potassium excretion in response to an acute potassium load and to furosemide. The latter is known to enhance potassium excretion by providing more sodium to the distal sodium-potassium exchange sites and by causing volume depletion with concomitant stimulation of aldosterone secretion (65). Since the aldosterone response to furosemide was normal in sickle cell patients, the blunted kaliuretic response cannot be explained on this basis. Although it would appear that this blunted response is further evidence for a tubular defect our data also demonstrated a blunted natriuretic response to furosemide in sickle cell patients. Therefore, the impaired response of U_KV post furosemide in the sickle cell patients could be partially explained by the decreased delivery of sodium to the distal potassium exchange sites.

Although patients with sickle cell disease manifest a defect in renal potassium excretion, hyperkalemia is conspicuously absent. During the potassium chloride infusion, the sickle cell patients excreted only 28% of the potassium load within five hours compared to 61% in the controls, yet plasma potassium rose similarly in both groups. These results strongly suggest the presence of an extrarenal mechanism, either acute or chronic, of potassium adaptation in the sickle cell group. A similar mechanism of potassium adaptation has been postulated in patients with chronic renal failure who have been shown to excrete significantly more potassium in their stool than normals (66).

Other authors have suggested that extrarenal adaptation results from the translocation of potassium from extracellular to intracellular compartments. Alexander and Levinsky reported lower plasma potassium

in rats treated with a high potassium diet following an acute potassium load (67). Since lower plasma potassium levels in the rats on the high potassium diet could not be explained by increased renal potassium excretion, these authors concluded that enhanced tissue uptake of potassium was responsible for the potassium adaptation. Furthermore, they demonstrated that potassium adaptation was lost following adrenalectomy and could be restored following mineralocorticoid replacement. Although the aldosterone response to volume contraction and cortrosyn was normal in our sickle cell patients, our data do not exclude enhanced extrarenal tissue sensitivity to aldosterone. Our data also do not define the relative contributions of enhanced gastrointestinal secretion versus increased cellular (liver, muscle, etc.) uptake of potassium following intravenous potassium chloride administration. However, it seems unlikely that increased cellular uptake of potassium could be playing a primary role in chronic potassium adaptation, since eventually cellular sites of potassium sequestration would become saturated and hyperkalemia would ensue. Since renal potassium excretion is low, it must be assumed that these sickle cell patients have augmented gastrointestinal potassium losses.

Evidence has also accumulated implicating insulin in the extrarenal mechanism responsible for modulating serum potassium levels (68,69). Acute hyperkalemia stimulates insulin release, which in turn blunts the rise in serum potassium concentration by enhancing cellular uptake of potassium (70).

In summary, then, our data show that in response to KCl infusion patients with sickle cell disease excrete less potassium in the urine compared to controls, yet their plasma potassium levels

are similar. In order to determine whether these patients have developed chronic adaptive mechanisms for eliminating potassium (e.g. G.I. losses), or whether the infused potassium is transferred acutely into the intracellular compartment (mediated by insulin) further investigations must be carried out. These include balance studies to compare daily urinary potassium excretion in sickle cell patients with controls, as well as measurement of fecal potassium losses.

CONCLUSION

The results of the present study demonstrate severe distal and collecting tubular dysfunction in patients with sickle cell disease despite normal preservation of glomerular function. All sickle cell patients displayed impaired concentrating and acidifying ability and decreased ability to excrete potassium in response to a variety of stimuli known to enhance potassium excretion. The defect in potassium excretion could not be explained by hypoaldosteronism or decreased distal sodium delivery. Despite the impairment in renal potassium excretion, hyperkalemia was not present suggesting an extra-renal mechanism of potassium adaptation.

It is likely that the defects in tubular function in these patients result from the sickling of red blood cells within the vasa rectae with resultant thrombosis and disruption of the microvasculature. The eventual result is fibrosis which is most pronounced in the medullary and papillary areas. The tubular damage results in a "functionally papillectomized" kidney, characterized by impaired concentrating ability, urinary acidification and potassium excretion. Although impairment in potassium excretion does not present an immediate clinical problem, it is likely that with time progressive renal damage may ultimately predispose to clinically significant hyperkalemia. Finally, the results of this study suggest that the sickle cell kidney may be viewed as a model for other forms of interstitial nephritis, in which impaired urinary concentrating ability, renal tubular acidosis, and hyperkalemia are important physiologic features.

TABLE I

CLINICAL PROFILE OF SICKLE CELL PATIENTS

	Age	Diagnosis	Hematocrit	% Hb S
ED*	22	Hb SS	30	65
RB	27	Hb SC	33	50
JG*	37	Hb SS	27	52
SE	26	Hb SS	19	91
LC	21	Hb SS	25	73
AE	19	Hb S-thal	28	66
mean ±SEM	25±2.6		27.0±1.95	66.2±6.13

* ED and JG had received recent blood transusions

TABLE II

SERUM ELECTROLYTES, BUN and CREATININE PRIOR TO STUDY

	Serum Na (mEq/L)	Serum K (mEq/L)	Serum HCO_3 (mEq/L)	Serum Cl (mEq/L)	BUN (mg/dl)	Creatinine (mg/dl)
ED	141	4.0	25	103	6	.5
RB	137	4.5	25	102	9	.6
JB	142	3.7	24	103	10	.6
SE	136	3.7	22	100	7	.5
LC	137	4.0	25	104	10	.7
AE	140	3.9	24	103	11	.9
mean ±SEM	138.8±1.01	3.97±.12	24.17±.50	102.5±.56	8.83±.80	.63±.06

TABLE III

GLOMERULAR FILTRATION RATE AND RENAL PLASMA FLOW

	GFR C_{In} (ml/min)	RPF C_{PAH} (ml/min)	Filtration Fraction C_{In}/C_{PAH}
ED	175	1108	0.16
RB	112	717	0.16
JG	103	906	0.11
SE	133	778	0.17
LC	125	639	0.20
AE	83	604	0.14
mean ±SEM	122±13	792±77	0.16±0.01

TABLE IV

URINARY CONCENTRATING ABILITY IN RESPONSE TO WATER DEPRIVATION AND AQUEOUS VASOPRESSIN*

	U_{osm} after 12 hrs H_2O Deprivation (mosm/kg)	U_{osm} after 14 hrs (mosm/kg)	U_{osm} after 16 hrs + 500 mU ADH (mosm/kg)
ED	380	377	422
RB	407	414	440
JC	342	381	426
SE	381	389	402
LC	388	397	401
AE	434	413	441
Mean ±SEM	389±13	395±6	422±7

* See text for values for age matched controls

TABLE V

VENOUS pH, AND PLASMA BICARBONATE CONCENTRATIONS BEFORE AND AFTER NH_4CL LOADING

	Venous pH	Venous $(HCO_3)^-$ (mEq/L)
Sickle Cell Patients:		
Before NH_4Cl	7.39±.01 (7.35-7.41)	24±0.5 (23-25)
*After NH_4Cl	7.34±.01 (7.31-7.35)	19±.7 (17-22)
Controls:		
Before NH_4Cl	7.35±.01 (7.33-7.37)	26±.5 (24-28)
*After NH_4Cl	7.27±.01 (7.26-7.29)	20±.5 (19-22)

* Represents minimum value

TABLE VI

RENAL RESPONSE TO ACID LOADING

	Minimum Urine pH	Maximum $U_{TA}V$ (μEq/min)	Maximum $U_{NH_4}V$ (μEq/min)	$\frac{U_{NH_4}V}{U_{TA}V + U_{NH}V}$ x 100%
Sicklers:				
ED	5.71	44	51	54
RB	5.74	28	49	64
JG	5.32	35	52	60
SE	5.78	56	64	53
LC	5.23	48	57	54
AE	5.31	41	27	40
Mean ±SEM	5.52±.10	42±4	50±8	54±3
Controls:				
A	4.70	118	80	40
B	5.14	109	91	46
C	5.10	124	75	38
D	5.10	84	86	51
E	5.30	70	41	37
F	5.00	70	57	45
Mean±SEM	5.06±.02	96±11	72±8	42±2
P value	$p<0.01$	$p<0.001$	$p<0.05$	$p<0.02$

TABLE VII

RENAL RESPONSE TO $NaSO_4$ INFUSION

	Basal U_KV (μEq/min)	Maximum U_KV (μEq/min)
Sicklers:		
ED	43	59
RB	47	58
JG	42	82
SE	43	60
LC	34	53
AE	15	30
Mean ±SEM	37±5	57±5
Controls:		
A	130	282
B	68	199
C	92	223
D	115	170
E	51	116
Mean ±SEM	91±15	198±20
	$p<0.01$	$p<0.01$

TABLE VIII

CHANGES IN URINE AND PLASMA POTASSIUM FOLLOWING KCl INFUSION

	Baseline U_KV (μEq/min)	Maximum U_KV (μEq/min)	% of KCl load excreted at 5 hrs	Baseline Plasma K^+ (mEq/L)	Maximum Plasma K^+ (mEq/L)
Sicklers:					
ED	22	85	31	3.41	4.08
RB	42	119	23	3.70	4.13
JG	39	70	9	3.49	4.08
SE	27	129	43	3.64	4.58
LC	42	164	33	4.04	4.73
AE	28	114	26	3.80	4.45
Mean ±SEM	33±4	114±14	28±5	3.68±.09	4.34±.12
Controls:					
A	72	230	60	3.89	4.40
B	90	376	70	3.50	4.65
C	130	389	41	4.08	4.68
D	46	471	54	3.70	4.35
E	48	298	82	3.74	4.23
Mean ±SEM	77±15	353±41	61±7	3.78±.10	4.46±.11
p values	$p<0.01$	$p<0.001$	$p<0.01$	NS	NS

TABLE IX

URINARY SODIUM AND POTASSIUM RESPONSE TO FUROSEMIDE

	Baseline U_KV (μEq/min)	Maximum U_KV (μEq/min)	Baseline $U_{Na}V$ (μEq/min)	Maximum $U_{Na}V$ (μEq/min)
Sicklers:				
ED	25	88	150	538
RB	24	148	91	3078
JG	48	102	48	347
SE	10	72	90	586
LC	27	122	89	933
AE	24	62	62	328
Mean ±SEM	26±5	99±13	88±14	968±431
Controls:				
A	65	189	182	1882
B	40	300	53	2343
C	35	209	93	2058
D	40	258	79	1494
E	34	138	64	1775
F	23	93	52	1792
G	14	205	39	1138
H	30	211	43	1774
I	35	228	64	1270
Mean ±SEM	35±5	203±20	74±15	1725±125
p values	NS	p<0.001	NS	*p<0.10

* p<0.001 for 5 out of 6 patients with sickle cell disease compared to controls. Note hyperresponsiveness of RB.

TABLE X

PLASMA RENIN ACTIVITY AND PLASMA ALDOSTERONE IN RESPONSE TO VOLUME DEPLETION

	Baseline PRA (Ng/ml/hr)	Post Lasix PRA (supine) (Ng/ml/hr)	Post Lasix PRA + ambulation (Ng/ml/hr)	Baseline plasma aldosterone (Ng/100 ml)	Post Lasix plasma aldosterone (supine) (Ng/100 ml)	Post Laxis plasma aldosterone (+ ambulation) (Ng/100 ml)
ED	.3	4.98	7.98	7.4	31.5	45.3
RB	.04	2.19	3.40	9.5	20.8	16.0
JG	.87	4.77	16.23	8.8	19.8	218.0
SE	1.33	5.10	15.3	6.3	8.2	46.4
LC	.47	7.47	8.57	11.6	41.8	37.6
AE	1.45	16.0	33.0	11.1	23.6	104.1
Mean ±SEM	.74	6.75±1.97	14.08±4.26	9.1±.84	24.3±4.66	77.9±30.5

TABLE XI

RESPONSE OF PLASMA ALDOSTERONE AND CORTISOL FOLLOWING ADMINISTRATION OF ACTH (CORTROSYN)

	Baseline plasma cortisol (µg/100 ml)	Post ACTH plasma cortisol (µg/100 ml)	Baseline plasma aldosterone (ng/100 ml)	Post ACTH plasma aldosterone (ng/100 ml)
ED	12	25	11.2	14.2
RB	17	28	NA	5.9
JG	8	17	13.0	52.0
SE	9	23	4.9	10.6
LC	13	28	11.6	17.6
AE	11	27	13	118.5
Mean ±SEM	11.5±1.4	25±1.7	10.7±1.5	36.5±17.7

See text for values for age matched controls

LEGEND FOR FIGURES

Figures 1, 2, and 3. Urine pH, titratable acid excretion and ammonia excretion are plotted during the ammonium chloride study. The values for each of the sickle cell patients are plotted individually and the range of the controls is shaded.

Figure 4. Urinary potassium excretion (U_KV) is plotted during the $NaSO_4$ infusion. The solid circles connected by solid lines (●——●) represent the curves for the individual sickle cell patients. The open circles connected by broken lines (o---o) represent individual curves for the control subjects.

Figure 5. Urinary potassium excretion (U_KV) is plotted during the KCl infusion. The values for individual sickle cell patients are represented by solid circles and solid lines. The control subjects are represented by open circles and broken lines.

Figure 6. The values for plasma potassium are plotted during the KCl infusion. The mean values and the S.E.M. for the control period, and for subsequent hourly intervals are plotted for the sickle cell patients (solid circles, solid line) and the controls (open circles, broken lines).

Figure 7. Urinary potassium excretion (U_KV) is plotted during the furosemide study. The values for each of the sickle cell patients are represented by solid black circles connected by solid lines. The range of the control values is shaded.

Figure 8. Urinary sodium excretion ($U_{Na}V$) is plotted during the furosemide study. The values for each of the sicklers are represented by solid black circles connected by solid lines. The range of the control values is shaded.

FIGURE 1

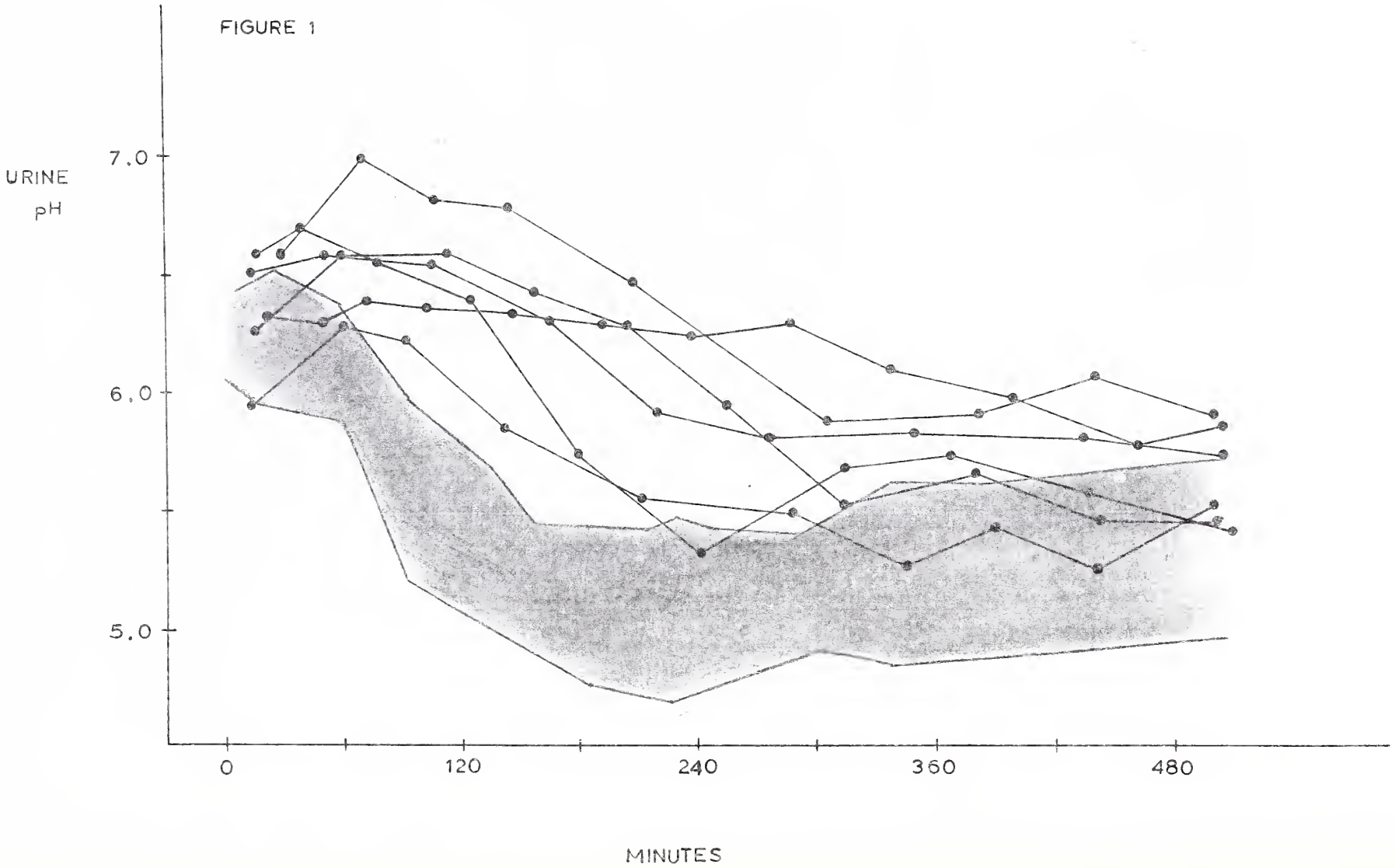


FIGURE 2

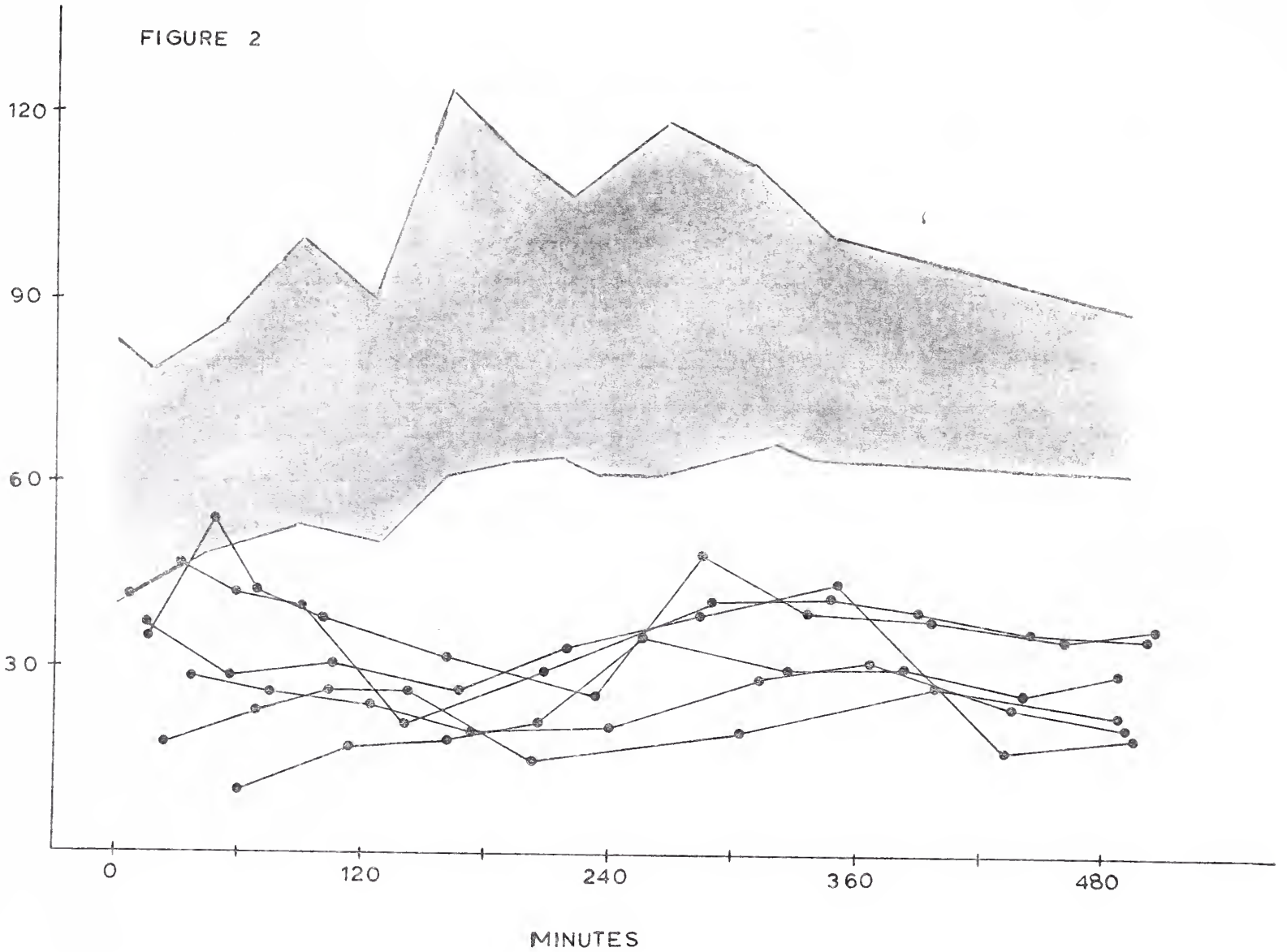


FIGURE 3

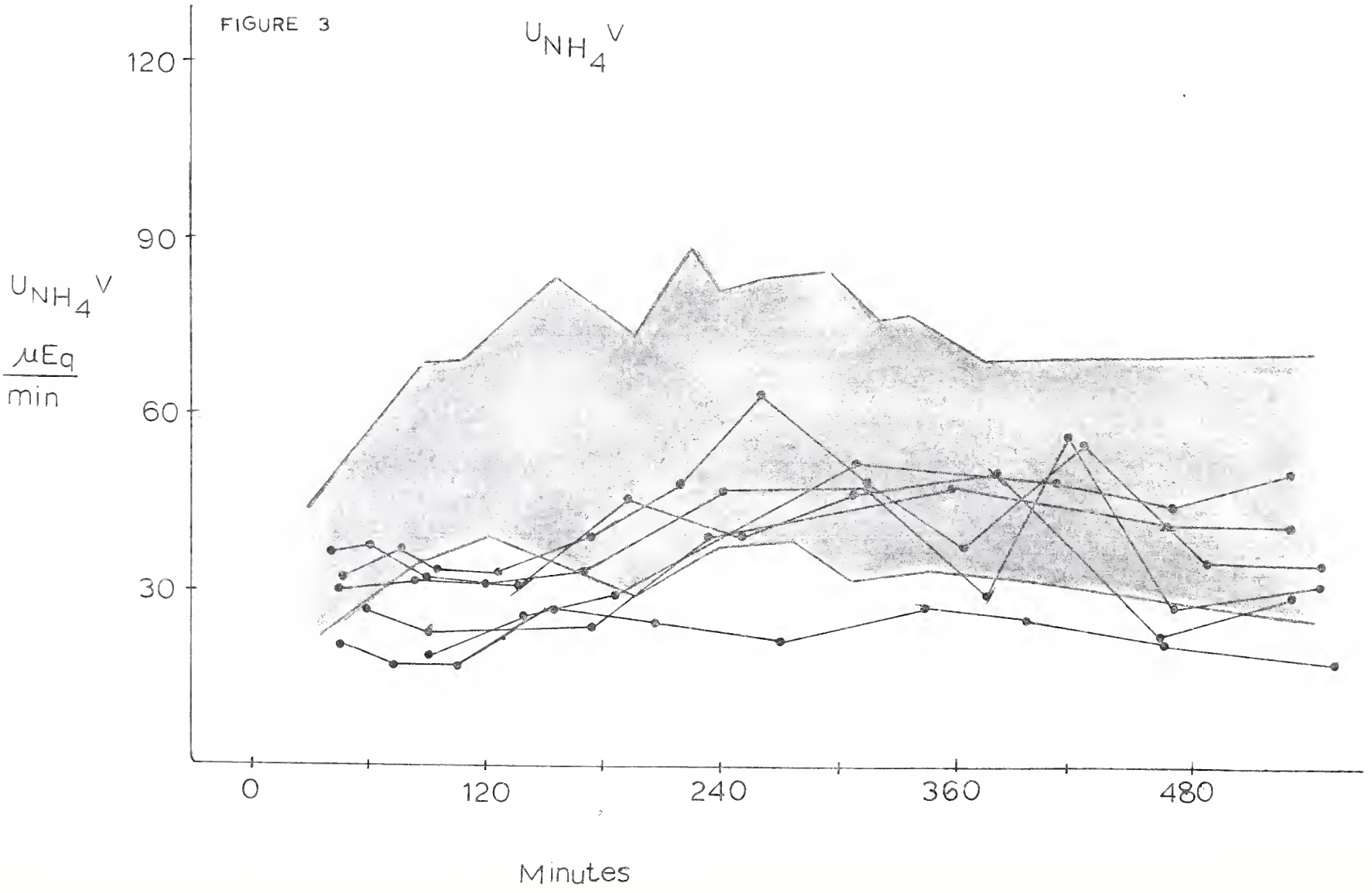
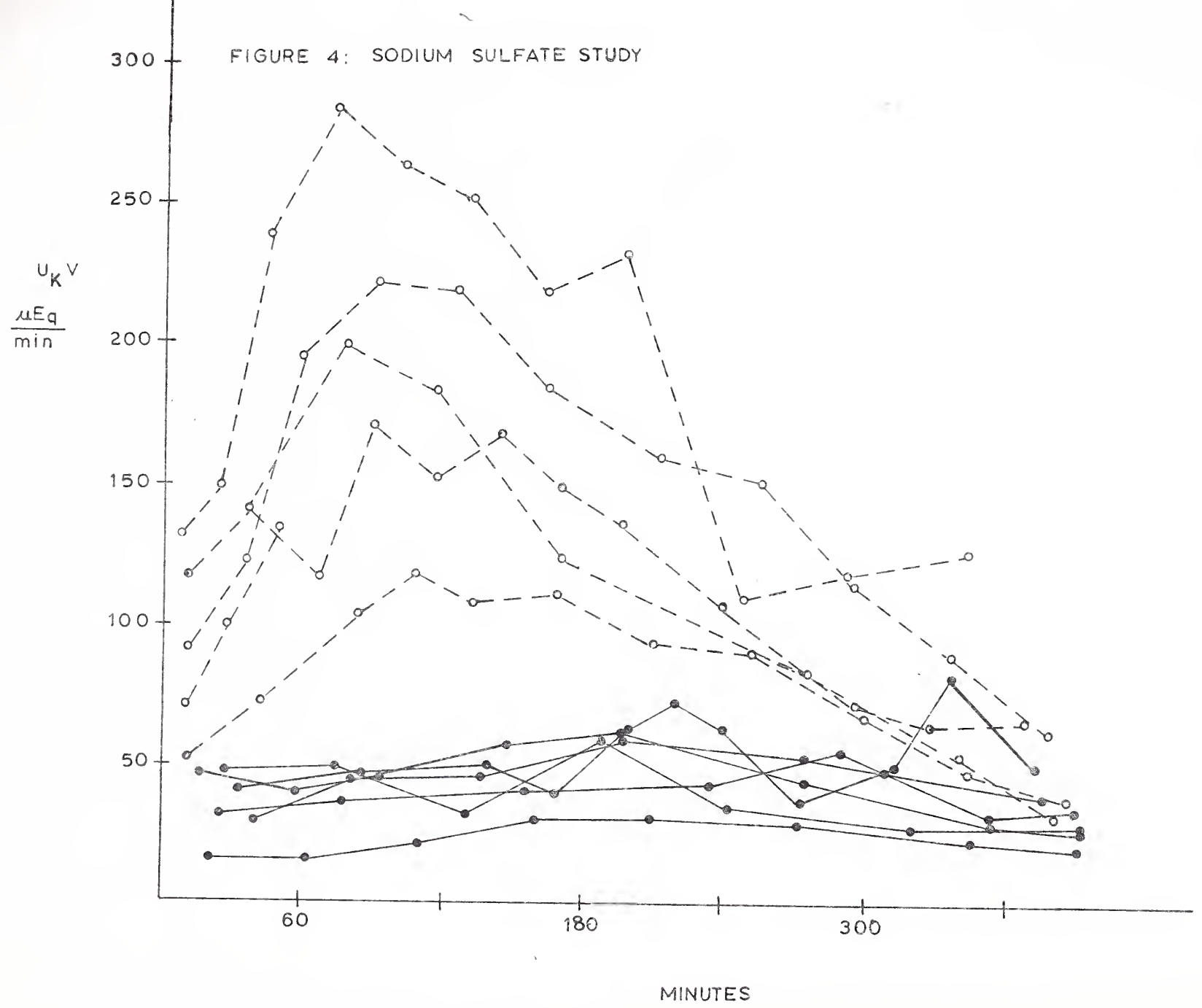

$U_{NH_4}V$
120
90
60
30
$U_{NH_4}V$
$\frac{\mu Eq}{min}$
0
120
240
360
480
Minutes

FIGURE 4: SODIUM SULFATE STUDY



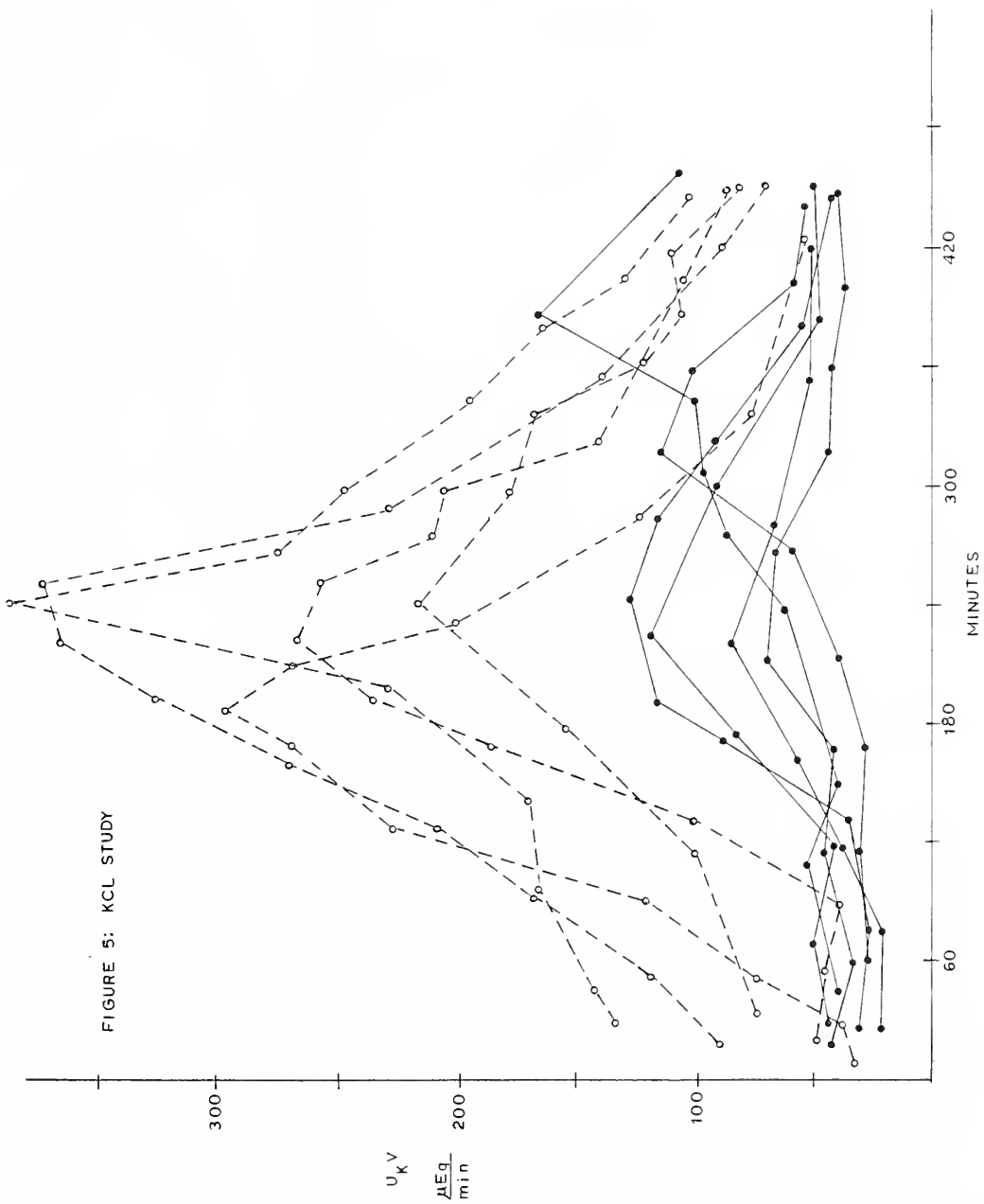


FIGURE 5: KCL STUDY

FIGURE 6

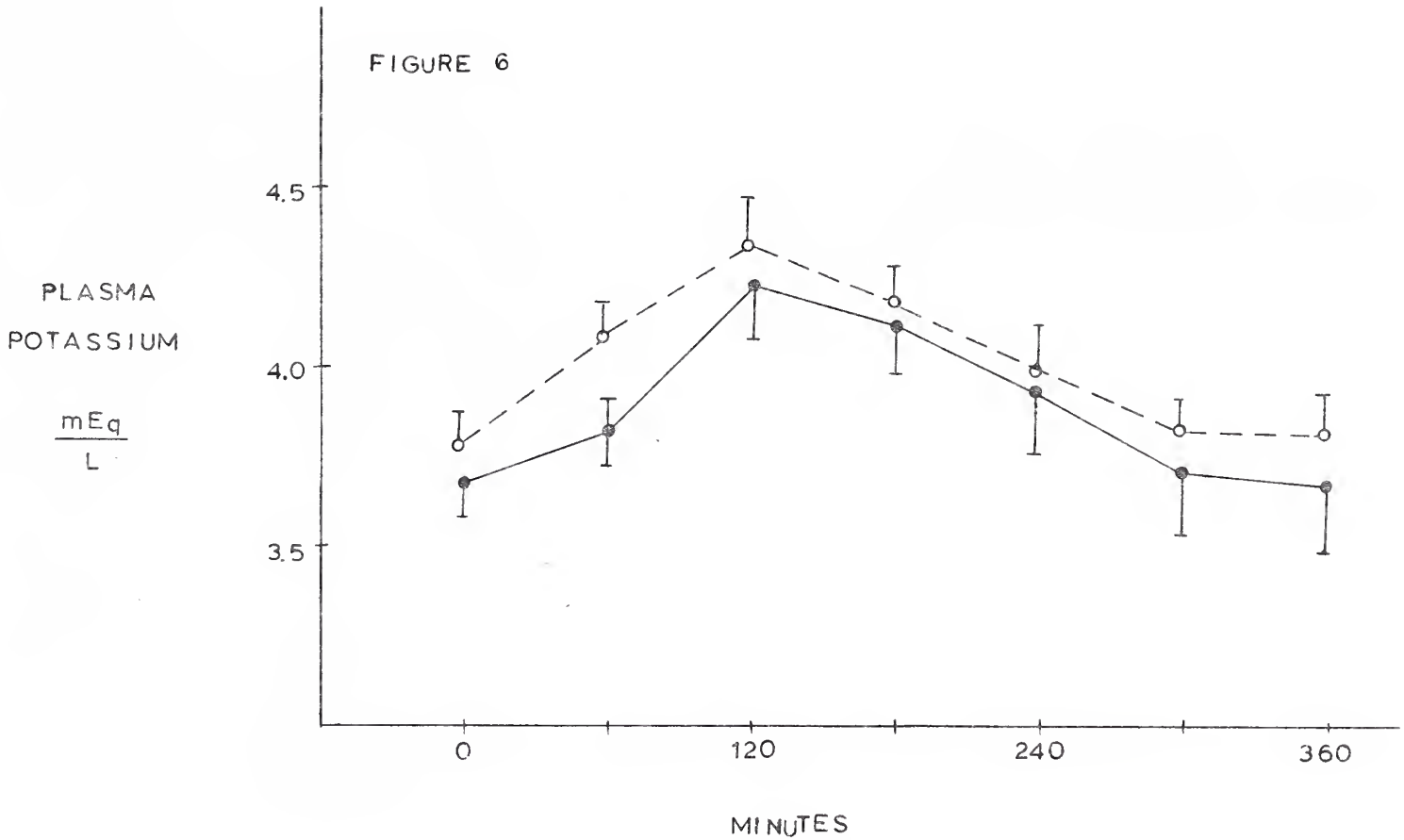


FIGURE 7

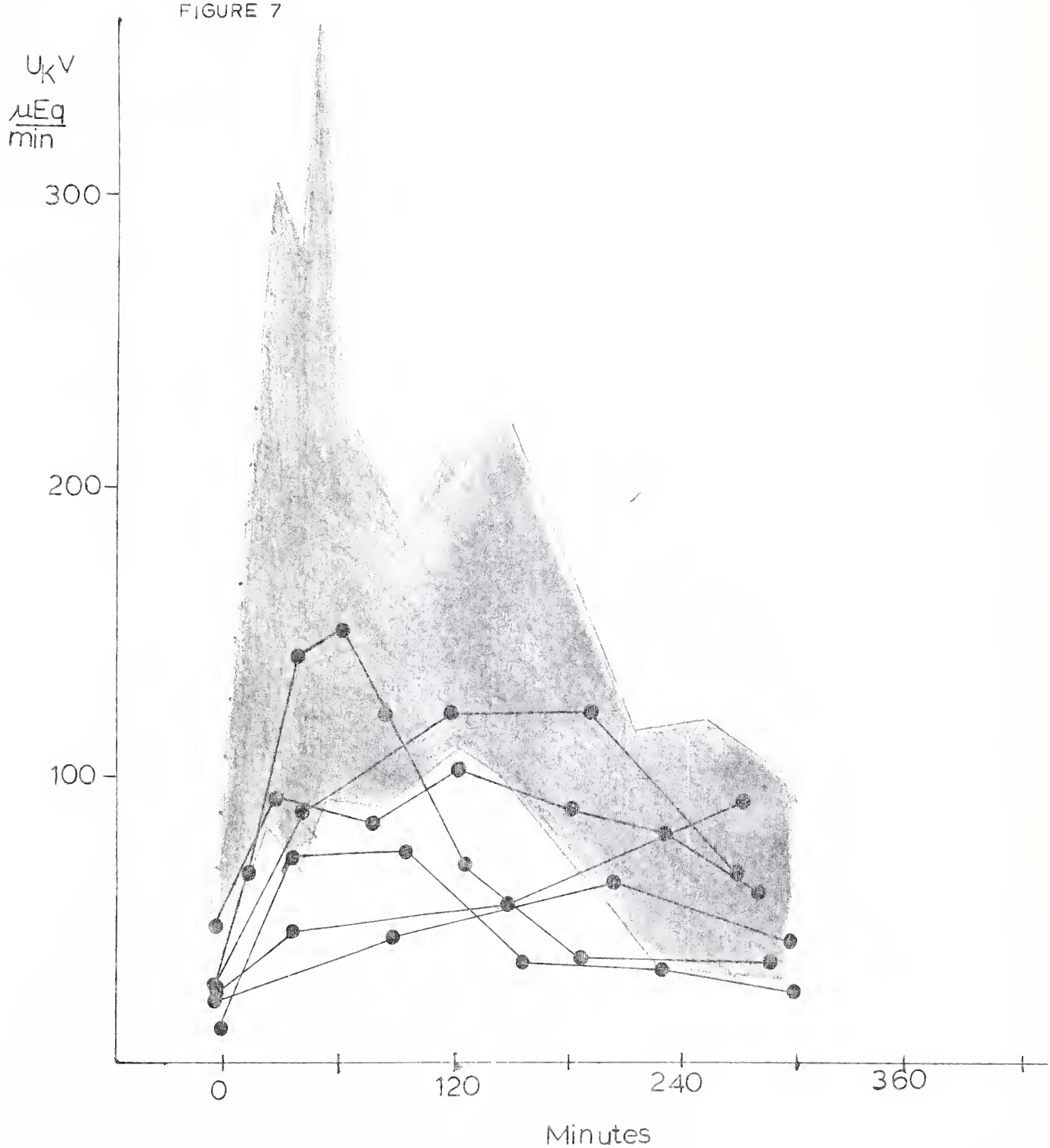
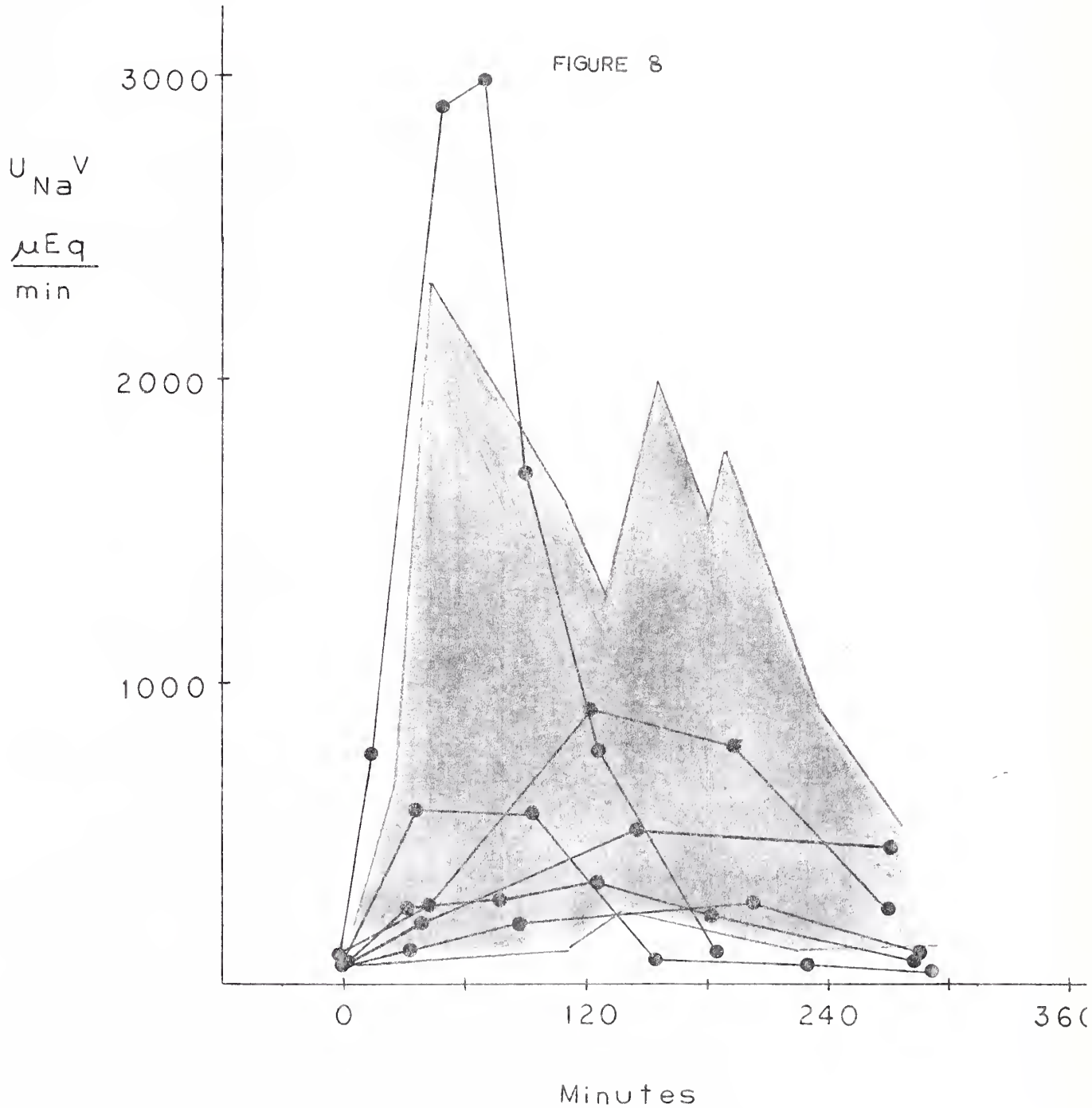


FIGURE 8



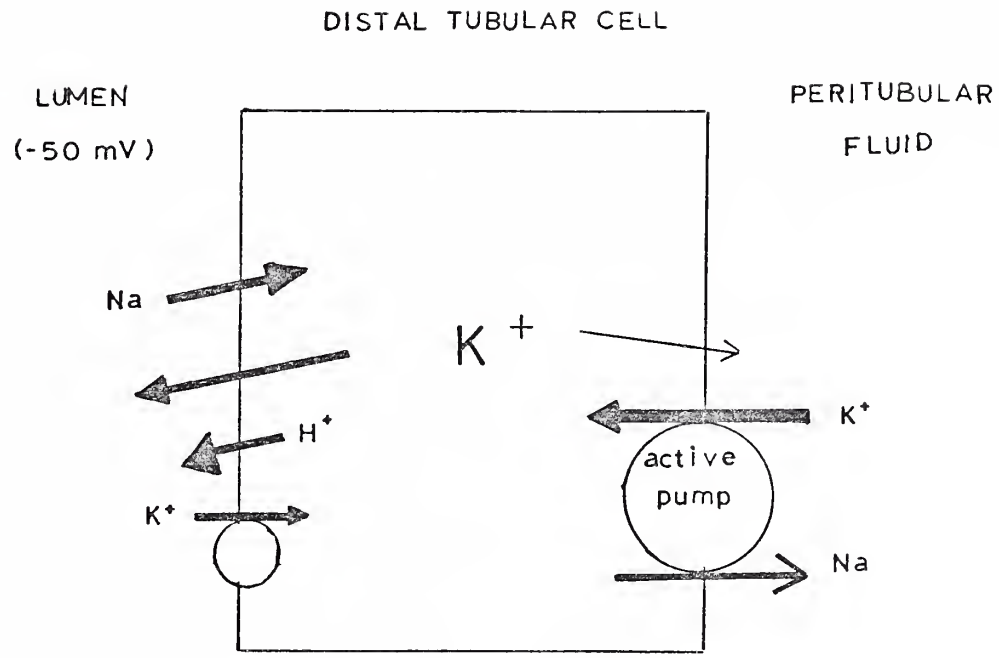


Figure IX: Schematic representation of potassium secretion by the distal tubular cell (see text).

Step 1: Intracellular potassium is maintained at a high level by an active pump located on the pericapillary side of the distal and collecting tubular cells.

Step 2: The passive diffusion of potassium from cell to lumen is also favored by the electrical gradient, lumen 50 millivolts negative with respect to cell.

Step 3: Since sodium permeability is greater than that of chloride, reabsorption of sodium causes the lumen to become slightly electronegative, and this in turn serves as a driving force for potassium secretion.

APPENDIX

Case Histories

ED: This 22 year old black male was first diagnosed as having Hb SS when he presented at age 3 with a pneumococcal pneumonia. Since that time he has had approximately seven episodes of pneumonia responding favorably to antibiotics. During his childhood and early adolescence the patient had approximately one painful crisis every three years. In the last year he has had 5 painful crises characterized by severe joint, abdomen, and chest pain, and has been hospitalized on 3 occasions. From age 3-9 years he received periodic transfusions to maintain his hemotocrit above 30 volumes percent and over the last year a similar transfusion regimen was reinstituted. His course has been complicated by recurrent bilateral medial malleoli ulcers treated with skin grafts. In 1975 the patient was noted to have cholelithiasis which was treated with cholecystectomy. There is no clinical history of cardiac or renal disease. On physical examination the blood pressure was 115/80 mm Hg, pulse 65 per minute, and respirations 14 per minute. The lungs were clear to auscultation and percussion. Examination of the heart revealed a II/VI systolic ejection murmur at the apex. Abdominal exam was unremarkable except for an old cholecystectomy scar; there was no organomegaly or tenderness. The extremities revealed healing ulcers over both medial malleoli. The remainder of the physical examination was within normal limits. Admission laboratory values included: hematocrit 30% with 65% Hb S (one week post transfusion); white blood cells 12,700/mm^3; and reticulocyte count 14%. Urinalysis revealed no protein, glucose, ketones, or cellular elements. Serum uric acid was 7.2 mg/100 ml. Serum electrolytes, blood urea nitrogen, creatinine, and inulin clearance are given in Tables I and II. Chest x-ray showed an enlarged heart with increased pulmonary flow. Electrocardiogram demonstrated left ventricular hypertrophy.

RB: This 27 year old black female was diagnosed as having HB SC at age 7 when she was evaluated for recurrent episodes of bone pain. She continues to have 1 to 3 attacks per month but these rarely result in hospitalization. She has not received any blood transfusions for the last seven years and she has never had skin ulcers, pneumonia, or clinical evidence of cardiac or renal disease. On physical examination the blood pressure was 120/90 mm Hg, the pulse 80 per minute, and respirations 16 per minute. Cardiopulmonary exam was within normal limits. The abdomen was non tender and there was no organomegaly. The remainder of the physical examination was within normal limits. Admission laboratory values were: hematocrit 32% with 50% Hb S; white blood cells 10,700/mm^3; and reticulocyte count 4%. Urinalysis revealed no protein, glucose or ketones, and 4-6 white blood cells per high power field. Serum uric acid was 5.1 mg/100 ml; and serum electrolytes, blood urea nitrogen, creatinine, and inulin clearance are given in Tables I and II. Chest x-ray revealed normal heart size, clear lung fields, and bony changes consistent with sickle cell disease. Electrocardiogram was within normal limits.

JG: This 36 year old black female was diagnosed as having Hb SS at age 22 during a routine evaluation for normal pregnancy. Her clinical course was unremarkable with only a few painful crises over the next 10 years, until 1972 when she presented with fever, weight loss, cough, and dyspnea on exertion. Chest x-ray revealed hilar adenopathy and conjunctival biopsy confirmed the diagnosis of sarcoid. The patient was treated with a short course of prednisone with complete resolution of her clinical symptoms and chest x-ray. Renal evaluation at this time revealed a normal serum creatinine and urinalysis. Since 1972 the patient has had approximately 4 painful crises per year characterized by abdominal, chest and joint pain and in 1974 she was placed on a hypertransfusion regimen, receiving 2 units of packed red blood cells every month. There is no history of recurrent infection, skin ulcers, or cardiac disease. Current medications include prednisone, 12.5 mg every day. On physical examination the blood pressure was 110/60 mm Hg, pulse 60 per minute, and respirations 14 per minute. The lung fields were clear to percussion and ausculatation. The cardiac exam revealed a soft S_4 at the left sternal border and a 2/6 systolic ejection murmur at the left sternal border. The spleen was not palpable. The remainder of the physical examination was within normal limits. Admission laboratory values were: hematocrit 29% with 52% Hb S; white blood cells 14,200/mm^3; and reticulocyte count 9%. Urinalysis revealed no protein, glucose, ketones, or cellular elements. Serum uric acid was 2.5 mg/100 ml. Chest x-ray revealed clear lung fields and a prominent left ventricle. Electrocardiogram was within normal limits.

SE: This 26 year old black female was diagnosed as having Hb SS at the age of 15 months when she presented with swollen, tender lower extremities. Since that time the patient's clinical course has been relatively benign with only 3 hospitalizations for painful crisis and one for pneumonia. In 1971 the patient became pregnant and was placed on a hypertransfusion regimen. However, she has not received any blood transfusions since her pregnancy in 1971. There is no clinical history of cardiac or renal disease. On physical examination the blood pressure was 124/72 mm Hg, the pulse was 88 per minute, and respirations 14 per minute. Physical examination was within normal limits. Admission laboratory values were: hematocrit 19% with 91% Hb S; white blood cells 12,500/mm^3, and reticulocyte count 17.2%. Urinalysis revealed no protein, glucose, ketones, or cellular elements. Serum uric acid was 4.3 mg/100 ml. Serum electrolytes, blood urea nitrogen, creatinine, and inulin clearance are given in Tables I and II. Chest x-ray was unremarkable except for bony changes in the vertebral bodies consistent with sickle cell disease. The electrocardiogram was within normal limits.

LC: This 21 year old black male was diagnosed as having Hb SS in early childhood when he presented with painful bone crisis and leg ulcers. Since this time he has had approximately 2-4 hospital admissions per year for painful crisis and was treated with blood transfusions on most of these occasions. Over the last two years he has noted mild dyspnea on exertion. There is no history of pneumonia, leg ulcers, or renal dysfunction. On physical examination the blood pressure was 110/60 mm Hg, pulse 70 per minute, and respirations 16 per minute. Examination of the eyes revealed mild scleral icterus. There were diffuse rhonchi heard during inspiration and expiration throughout both lung fields. The heart sounds were normal and no murmurs were present. The liver span was 14 cm; the spleen was not palpated. The remainder of the physical examination was within normal limits. Admission laboratory values were hematocrit 25% with 73% Hb S; white blood cells 12,400/mm^3; and reticulocyte count 9%. Urinalysis revealed no protein, glucose, ketones, or cellular elements. Serum electrolytes, blood urea nitrogen creatinine and inulin clearance are given in tables I and II. Serum bilirubin was 0.25 mg/100 ml indirect, and 4.0 mg/100 ml total and liver function tests were all normal. Chest x-ray showed mild cardiomegaly and bony changes consistent with sickle cell disease. Electrocardiogram was within normal limits.

AE: This 19 year old black female was first diagnosed as having Hb S-thal at birth. She was hospitalized only once as a child for a painful bone crisis. Over the last 5 years she has had frequent (3-4 per month) episodes of joint and bone pain which she treats at home with bed rest, tylenol, and fluids. In the last two years the patient has been hospitalized on two occasions for gross hematuria and bacteriuria. On both occassions the hematuria resolved with fluids and antibiotics. She has not had any further episodes of hematuria, urinary tract infection, pyuria, or bacteriuria. She has only been transfused on one occasion. There is no history of leg ulcers, pneumonia, or cardiac disease. On physical examination the blood pressure was 114/70 mm Hg, pulse 80 per minute, and respirations 16 per minute. Cardiac exam revealed a grade II/VI systolic ejection murmur at the lower left sternal border. On abdominal exam the spleen was palpated 2.5 cm below the left costal margin. The liver was not enlarged. The remainder of the physical examination was within normal limits. Admission laboratory values were hematocrit 25% with 66% Hb S; white blood cells 6,500/mm^3, and reticulocyte count 23%. Urinalysis revealed no protein, glucose ketones, or cellular elements. Uric acid was 6.5 mg/100 ml. Serum electrolytes, blood urea nitrogen, creatinine, and inulin clearance are given in Tables I and II. Chest x-ray was normal. Electrocardiogram revealed inverted T waves in the precordial leads. An intravenous pyelogram done on this admission was entirely normal.

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